Docket No.: TDT-ADV-006 (TJU-2441)   **PATENT**
Serial No. 09/820,215   **Filed: March 27, 2001**

IN THE SPECIFICATION

Please replace paragraph on page 7, lines 4-16 as follows:

—Figure 3 is a photograph of an ethidium bromide-stained gel showing nested RT-PCR products, representing the expression of GC-C mRNA in peripheral blood mononuclear cells enriched in CD34+ cells from a patient undergoing peripheral blood progenitor cell harvest following treatment with ~~Neupogen~~ Neupogen™ (G-CSF; Amgen; Thousand Oaks, Calif.). For PBPC mobilization in preparation for bone marrow ablation and autologous transplantation, a patient with breast cancer received 10 µg/Kg/day (total daily dose of 600 µg) of Neupogen™ (G-CSF; Amgen; Thousand Oaks, Calif.)as an IV bolus for 3 consecutive days. Leukaphoresis was performed on days 3 and 6 following the first dose of Neupogen™. Total RNA (1 µg) extracted from the mononuclear cells obtained on days 3 and 6 was subjected to RT-PCR employing GC-C-specific primers. The patient had a leukocyte count of 107,000/µl on day 3 and 17,000/µl on day 6. T84 cells served as a positive control for GC-C expression. H_2O (negative control) indicates no input RNA. Molecular weight markers and their sizes are indicated in the left lane. The arrow at the right indicates the size of the human GC-C RT-PCR product (~250 bp) predicted from the defined sequence.--

Please replace paragraph on page 23, lines 9-21 as follows:

--   Blood and tissue specimens were obtained from the hematology/oncology clinic under an Institutional Review Board-approved protocol (Control #98.0614) at Thomas Jefferson University Hospital (Philadelphia, Pa.) and the Cooperative Human Tissue Network (Philadelphia). Healthy volunteers and Dukes' Stage D patients were informed about the study and asked to participate. After informed consent was obtained, each participant received a unique identification number that was recorded on blood samples and any acquisition forms. Blood (~16 cc) collected into Vacutainer® CPT™ tubes (cell preparation tube) containing sodium heparin was centrifuged at 25°C. for 15 minutes at 1700 rpm and the resulting mononuclear cell, red blood cell, and granulocytes fractions recovered for RNA extraction. In some experiments, whole blood was centrifuged at 1300 rpm at 4°C. for 10 minutes, the resulting supernatant containing the platelet-rich

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plasma was centrifuged at 3000 rpm at 4°C. for 10 minutes, and the platelet pellet was recovered for RNA extraction.—

Please replace paragraph beginning on page 24, line 25 and ending on page 25, line 19 as follows:

--   The expression of epithelial cell markers in blood cells was examined by RT-PCR employing transcript-specific primer sets (Table 1). Reverse transcription of total RNA (≤1 µg) was performed with 0.25 units/µl of AMV reverse transcriptase (Panvera; Madison, Wis.) and buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM KC1, 4 mM $MgCl_2$, 1 mM each of dATP, dCTP, dGTP, and dTTP, 1 unit/µl RNase inhibitor (Panvera; Madison, Wis.), and 1 µM of the appropriate antisense primer in a total volume of 20 µl. Thermal cycling proceeded for 1 cycle at 50° C. for 30 minutes, 99° C for 5 minutes (to inactivate reverse transcriptase), and 4° C for 5 minutes. The resultant cDNA was subjected to PCR in the same reaction tube and included 2.5 units of TaKaRa Taq polymerase (Panvera; Madison, Wis.) in 10 µl of: 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, and 0.2 µM of the appropriate sense primer. Incubation and thermal cycling conditions were: 95° C. for 2 minutes, 1 cycle; 94° C. for 30 seconds, 58° C. for 30 second, 72°. C. for 90 seconds, 35 cycles; 72°. C. for 5 minutes, 1 cycle. Following RT-PCR, samples were stored at -4° C ? until analysis. Nested PCR (70 cycles) was performed employing 5% of the PCR product (DNA) and 2.5 units of TaKaRa Taq polymerase (Panvera; Madison, Wis.) in 100 µl of: 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, and 0.2 µM of the appropriate sense primer. Incubation and thermal cycling conditions were: 95° C. for 2 minutes, 1 cycle; 94° C. for 30 seconds, 58° C. for 30 seconds, 72° C. for 90 seconds, 35 cycles; 72° C. for 5 minutes, 1 cycle. Amplicons were separated by 4% Nusieve 3:1 agarose (FMC Bioproducts; Rockland, Me.) and visualized by ethidium bromide. Amplicon identity was confirmed at least once by DNA sequencing. RT-PCR was performed utilizing primers for β-actin on all samples to confirm the integrity of RNA. RNA extracted from T84 human colon carcinoma cells was employed as a positive control for GC-C mRNA. Negative controls included RT-

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PCR incubations that omitted RNA template. Primers employed for GC-C amplification span predicted intron-exon junctions, reducing the probability that amplification products reflect contaminating DNA templates.--

Please replace paragraph on page 27, lines 3-13 as follows:

--Monocytes, NK cells, T cells, CD19+ B cells, and CD34+ progenitor cells were obtained commercially (Bio-Whittaker; Charlotte, N.C.). Purified NK and T cells were generously provided by Dr. Bice Perussia, Kimmel Cancer Institute, Thomas Jefferson University. CD34+ progenitor cells were isolated from peripheral blood with a CD34 progenitor cell isolation kit. the (CD34 Progenitor Cell Isolation Kit™ (Miltenyi Biotec; Bergisch Gladbach, Germany). Similarly, populations of mononuclear cells were depleted of CD34+ cells by use of this kit. CD34+ progenitor cells were indirectly magnetically labeled using hapten-conjugated primary monoclonal antibody directed to CD34 and an anti-hapten antibody, coupled to MACS™ microbeads (MACS™ microbeads Bio-Whittaker, Charlotte, N.C.). Magnetically labeled cells were purified and recovered in the magnetic field of a MACS™ separator (MACS™ Separator™ Bio-Whittaker, Charlotte, N.C.).--

Please replace paragraph on page 27, lines 15-17 as follows:
-- All reagents were of analytical reagent grade. Results are representative of at least three experiments. Values representing the mean ± SD were calculated using computer software (Microsoft Excel™).--

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